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A Histologic Study of Healing of Split Thickness Gingival Flap Surgery in Dogs

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A HISTOLOGIC STUDY OF HEALING OF
SPLIT THICKNESS GINGIVAL FLAP SURGERY
IN DOGS

by
Harry Staffileno, Jr.

A Thesis Submitted to the Faculty of the Graduate School
of Loyola University in Partial Fulfillment of
the Requirements for the Degree of
Master of Science

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LIFE

Harry Staffileno, Jr. was born in Wellsburg, West Virginia, November 11, 1930.

He attended elementary schools in Wellsburg and graduated from Wellsburg High School in 1948. He attended West Virginia University and received a Bachelor of Arts Degree in June 1952. In September of the same year he entered Loyola University School of Dentistry and was conferred the Doctor of Dental Surgery Degree in June 1956.

After graduation from the school of dentistry, he received a post-graduate fellowship in Periodontics. He completed a two-year program for specialty certification in Periodontics in June 1958. At that time he received an appointment as Research Associate in the Department of Periodontics.

The author is an active member of component local, state, and national dental societies. He is also a member of Sigma Xi, International Association for Dental Research, and the American Association for the Advancement of Science.

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CHAPTER I

INTRODUCTION

In the literature clinical observations and histologic studies of the healing of repositioned mucoperiosteal flaps have been frequently reported. However, careful studies of the specific reaction of bone, of connective tissue, and of epithelium in split thickness gingival flaps have not been reported in detail. There is a need for a careful delineation of the phasic processes of healing in the split thickness flaps, since more recently an increase in use of this type of surgical procedure has been advocated in human periodontal therapy. This investigation was undertaken to study the tissue reactions in this type of flap operation.

11-11-51 S. L. H. Jones, Jr.

CHAPTER II

REVIEW OF THE LITERATURE

I. Introduction

Many surgical procedures are performed which require access to anatomical structures beneath the oral mucosa. Most often this is facilitated by raising a mucoperiosteal flap which serves as a soft tissue covering that is reopposed at the completion of the operation.

A review of the literature reveals several studies relative to the histology of healing of a mucoperiosteal flap. Some of the studies were done on human subjects and other investigations were carried out with dogs.

The term mucoperiosteal flap refers to a surgical flap of tissue consisting of one-half of the papillary gingiva, as well as the marginal, attached and unattached gingiva with the underlying periosteum. This is severed from the necks of the teeth and the alveolar bone by two vertical incisions at the mesial and distal borders of the flap. The only variation in this flap design was due to some authorities who showed a reluctance to sever the marginal gingiva from the teeth. In those cases, a horizontal incision was made below the margin of the gingiva to connect with the two vertical incisions.

Some studies were of the clinical healing while others observed the clinical and histologic healing.

II. Clinical Studies

Dingman (1947) did several studies of healing of mucoperiosteal flaps on humans. He stated that it was perfectly permissible to detach the gingival

tissues from around the necks of the teeth. When these flaps were carefully replaced, they reattached and in seven to ten days it was impossible to detect clinically that the gingival attachments had been disturbed.

Much attention in these healing studies of mucoperiosteal flaps was focused on the attachment of the gingiva to the tooth.

It should be noted that at least two different views exist on the relationship between the crevicular epithelium and the enamel or cementum or both. Orban (1952) stated that there is an organic union of the epithelium in attachment to the tooth. Waerhaug (1952) feels that his investigations prove that it is not possible to distinguish microscopically between tissue attachment and intimate contact.

III. Histologic Studies

Upon removing and studying block sections of surgically detached tissue from the maxilla and mandible in humans, Workman (1947) concluded that four weeks post-operatively, the relationship between the tooth and periodontal membrane was restored to its preoperative status.

Borden (1948), by a series of measurements from a study of twenty-five mucoperiosteal flaps in dogs and humans, reported that a reattachment of the detached tissue to the tooth does take place. However, he noticed that the depth of the gingival sulci around the operated teeth decreased due to a recession of the gingival tissue. Also a decided V-shaped loss of tissue existed at the gingival extremity of the vertical incision -- the extent of the defect varying inversely with the thickness of the incised tissue. From his clinical observations, the author concluded that a surgical flap, whenever

possible, be reflected so as not to involve the marginal gingiva of the adjacent teeth. The histologic examination showed that: (1) Connective tissue fibers of the periodontal membrane will reattach themselves to the cementum. (2) Granulation tissue formed in the periodontal space with subsequent fibrosis at the site of separation. (3) New cementum, bone, and connective tissue fibers are not essential for reattachment.

Svoboda (1947) operated mucoperiosteal flaps in two humans and concluded that the area was completely healed, clinically, in twenty-two days. Histologically, however, thirty-one days were required to have complete repair.

A histologic study of mucoperiosteal flap healing in six human subjects by Dedolph and Clark (1958) showed that the epithelial attachment was restored to its preoperative status in twenty-one days. Within the same time period the periodontal fiber bundles and the other connective tissue elements were restored and an inflammatory response at that time was mild or absent. The clinical appearance of the flap area was indistinguishable from that of the control specimen at the end of this three week period.

In reviewing the various studies reported, there seems to be some conclusions drawn on clinical measurements only, while other investigators used clinical and histologic evidence in their conclusions.

IV. Related Studies

Grant and Ivancie (1957-1958) determined the differences in the replacement tissue after gingival repositioning operations. The alveolar mucosa with its elastic fibers was replaced with collagenous connective tissue in eight months. They found epithelial ridge formation, keratinization and

orientation of the mature collagen fiber bundles. However, the result was that this new tissue was less differentiated and functionally immature gingiva.

A study by Mittelman (1958) on primary wound healing of a 1.5 mm. stab wound in the attached gingiva showed that epithelial regeneration is more rapid than connective tissue regeneration. Even though the wound was covered by epithelium in twenty-four hours, inflammation subsisted in the connective tissue as long as seventy-two hours.

Mucogingival surgery by Wilderman (1959) consisted of exposing the alveolar bone in dogs by a gingival reposition operation. The results showed a deficiency of repair of the vestibular bone so that the fibrous connective tissue attachment increased to compensate for this lack of bone. Also the epithelial attachment would not reattach at the cemento-enamel junction. Instead, reattachment occurred in a more apical position on the root surface. The author concluded that the dento-gingival junction exhibited a functional repair but with an anatomical deformity.

A study of ten young adult dogs on whom 6 x 7 mm. of crestal bone was removed in a flap operation was done by Marfino (1958). A medicated pack was placed between the flap and the tooth and removed after three weeks. Results showed a new connective attachment of 1.25 to 2.50 mm. Atrophy of the gingiva decreased the depth of the postoperative pocket by one-third. A small amount of new bone was observed at the alveolar crest but without total regeneration of the original morphology.

Regeneration of the gingiva in monkeys was studied by Cohen (1960). A gingivectomy was performed in the region of the premolar teeth and the soft

tissue below the area of gingivectomy was dissected from the bone and moved apically. It took six weeks for complete epithelialization. The results showed that the area of attached gingiva was increased and the muco-gingival junction was more apically positioned.

Heretofore, no author has reported a study of repair of a split thickness gingival flap. By definition this operation would involve a flap consisting of one-half of the papillary gingiva as well as marginal, attached and unattached vestibular gingiva, dissected from and not stripped from the tooth and bone. Dogs are preferable in such a study because of good access and control. The healing periods before sacrifice could be so selected on the basis of previously reported material, to observe maximum activity of the repair phenomenon. Our problem would entail the timing, where, how and the rate of repair of the tissue. Secondly, how much time necessary for this split thickness flap to heal before its histologic appearance coincides with that of the control or normal.

CHAPTER III

MATERIALS AND METHODS

This investigation was conducted on four adult dogs with completely erupted permanent teeth. The dogs were approximately two years in age.

Surgery was performed on these animals involving the border of free gingiva, attached gingiva and the alveolar mucosa in the buccal region of the premolar teeth in the maxilla and mandible (Plate I, Fig. 1). Each surgical procedure involved only one quadrant of either jaw at a time. The operation consisted of two vertical incisions which were made from the free gingival margin into the vestibular mucosa. One incision was placed mesially to the first premolar while the other was made distal to the third premolar. All vertical incisions penetrated the fibrous periosteum. A third incision was made into the gingival sulcus to split the fibrous connective tissue attached to the tooth. The size of the flap was approximately 10 mm. by 36 mm. (Plate II, Fig. 3).

This then provided the outline of a flap which was made up of one-half of the papillary gingiva, as well as marginal, attached, and unattached vestibular gingiva. This flap was delicately dissected from the attached underlying tissues so as to leave a stump of tissue attached to the tooth and to the periosteum (Plate II, Fig. 3, 4). The dissection was made in the deep layers of the lamina propria. Heretofore, this flap will be referred to as a split-thickness gingival flap. The clinical base of the flap was the vestibular mucosa.

Once this flap was operated, it was repositioned and sutured to place with 000 black silk suture (Plate III, Fig. 5). All sutures were removed in

six days in animals whose experiment extended beyond that time.

As a fixed clinical reference for the margin of the free gingiva, a notch was made into the surface of the enamel prior to operating the flap.

The surgery was performed under general anesthetic using one-half cc of sodium nembutal per pound of animal weight.

The only medication postoperatively was an immediate intramuscular injection of 900,000 units of penicillin.

The animals were fed on a diet of pulverized meal throughout the experiment.

Kodachrome clinical photographs were taken of the experimental area at various times to record clinical impressions. The animals were sacrificed by an overdose of nembutal injected directly into the heart.

After surgery sacrifice times of the animals were:

zero hours

two days

six days

fourteen days

twenty-one days

sixty days

The jaws of the sacrificed animals were then allowed to remain in a 10% formalin solution for two weeks.

The maxillary second premolar and the mandibular third premolar areas were considered central wound areas for histological examination.

Control specimens were taken from an unoperated area of one animal (Plate I, Fig. 2).

Histological examination of the operated areas was secured by the following method:

Fixation - 10% neutral formalin solution.

Decalcification - large quantities of a 5% aqueous nitric acid solution observed every two days until completely decalcified.

Specimen washed in running water for twenty-four hours, then neutralized in 10% formalin to which an excess of calcium or magnesium carbonate had been added. Again washed in water for twenty-four to forty-eight hours.

Dehydration	75% alcohol (twenty-four hours)
	95% alcohol (twenty-four hours)
	100% alcohol (twenty-four hours)
	Ether alcohol - 1/2 and 1/2 (twenty-four hours)

Embedded - celloidin

Thin - one week

Medium - one week

Thick - one week

Sectioned	Serially - oral vestibular
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Stained	Hematoxlin and eosin
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CHAPTER IV

1. MACROSCOPIC FINDINGS

A split thickness flap measuring 10 mm. wide by 36 mm. long was elevated and reflected from the vestibular plate of alveolar bone in the area of the premolar teeth (Plate II, Fig. 3). After surgery the flap was reopposed to the original position (Plate III, Fig. 5). The gingival border of the flap was sutured to the other half of the papillary portion of the lingual attached gingiva which was not severed. A clot formed at the edge of the wound and there was primary closure with little hemorrhage.

In two days the flap appeared swollen with a bright red line marking the area of vertical incision (Plate IV, Fig. 6). The gingival margin border of the flap had a rolled edge which folded over and into the incision.

At six days the vertical incision areas were still discernible by a linear depression in that area (Plate VII, Fig. 10). Clinical observation at this time indicated that the flap was attached to the underlying connective tissues (Plate VII, Fig. 11).

Within fourteen days post operative time, the flap area appeared healed except for the scar formations which were visible at the original borders of the flap (Plate XVI, Fig. 20). The tissue gave a clinical impression of being normal in every other aspect. There was never any change in the characteristic of pigmentation of the tissue.

In the twenty-one day animal and sixty day animal the flap region appeared the same as the control specimen (Plate XVII, Fig. 22, Plate XIX, Fig. 25).

2. MICROSCOPIC FINDINGS OF HEALING OF SPLIT THICKNESS FLAP

- I Initial or Preparatory Stage 0-2 days
 - A Bleeding and Clotting 0 hour
 - B Initial Fusion of Flap (Anchoring Clot) 0-48 hours
 - C Inflammation 0- $\frac{1}{2}$ hour
- II Productive Stage 2-6 days
 - A Epithelial Regeneration
 - 1 Epithelial Bridging
 - 2 Epithelial Proliferation and Maturation
 - B Connective Tissue Production
 - 1 Connective Tissue Fusion
 - 2 Connective Tissue Proliferation
 - C Inflammation
- III Reconstructive Stage 0-60 days
 - A Reconstruction of Connective Tissue
 - 1 Continued Connective Tissue Proliferation 14-21 days
 - 2 Connective Tissue Maturation 6-60 days
 - B Reconstruction of Bone
 - 1 Osteoclastic resorption 6-14 days
 - 2 Osteogenesis 6-60 days

MICROSCOPIC FINDINGS OF HEALING OF SPLIT THICKNESS FLAP

I Initial or Preparatory Stage 0-2 days

A. Bleeding and Clotting 0 hour

At zero hour the specimen of the operated animal shows a split thickness gingival flap in which the incision had extended through the surface epithelium and into the lamina propria so that about one-half of the latter formed the flap and the other half remained as a bed of connective tissue (Plate II, Fig. 4). The part of lamina propria and epithelium which remained attached to the tooth and attached to the periosteum overlying the vestibular plate of bone forms the stump. This portion of residual tissue includes the severed epithelial attachment which remains as a tag of tissue attached to the tooth surface.

A thin clot was found between the flap and the periosteal connective tissue.

The epithelium from the flap side appeared rolled in over the edge of the incision.

B. Initial Fusion of Flap (Anchoring of Clot) 0-48 hours

Within the few minutes following surgery and sacrificing the animal, an early fusion of the flap and stump with the opposing connective tissue is mediated by a clot. The clot consists of fibrin stroma into which is enmeshed many polymorphonuclear leukocytes, red blood cells, debris of injured cells from the connective tissue and capillaries of the edge of the wound. Bacteria and an exudate and transudate with tissue debris as a result of the tissue injury are present in this area at this time.

This matrix of clotted elements occupied the incision area, acting as a seal in fusing the flap with the attached connective tissue by means of the

fibrin network.

C. Inflammation 0 hour

An inflammatory response took place immediately following the surgery. Primarily, there was an acute influx of polymorphonuclear leukocytes in the blood clot.

On observing the lamina propria immediately below the epithelium in the gingival zone of the flap, dilated capillaries which were congested with blood elements were seen. The central portion of the capillaries was occupied by many red blood cells while the periphery showed a margination of the polymorphonuclear leukocytes. The connective tissue in this area contained many extravasated acute inflammatory cells.

In the supra alveolar zone (Plate XX, Fig. 28) of this flap the capillaries were also dilated and filled with blood cells with a higher ratio of polymorphonuclear leukocytes to the other cells. The number of polymorphonuclear leukocytes in the connective tissue in this area was smaller than the number of polymorphonuclear leukocytes in the gingival zone.

The Alveolar zone of the flap demonstrated dilated capillaries, and the connective tissue here showed the greatest number of polymorphonuclear leukocytes than the other two-thirds of the flap at the zero hour.

It was significant at this time that although the capillaries in the connective tissue adjacent to the periosteum and tooth were dilated, there was only an occasional polymorphonuclear leukocyte to be found in the extra vascular area.

II Productive Stage 2-6 days

A. Epithelial Production

1. Epithelial Bridging

Within forty-eight hours after surgery a break in the continuity of the epithelium over the incision area still existed. However, activity of the epithelium at this time indicated that bridging of the incision area was its main objective (Plate V, Fig. 8). From the flap side the epithelium was decreasing in dimension from the basal cell layer to the outer keratin layer. It may be described as a slipping or sliding of the cells over one another in an effort to stretch out across this defect over the clot.

Within six days the specimen presented a different picture. The epithelial bridging has been completed and further repair of the epithelium has also taken place (Plate VII, Fig. 11).

2. Epithelial Proliferation and Maturation

From the moment the epithelium has bridged over the incision area, there is a continuous proliferation of epithelial cells to quantitatively restore the quality of the tissue in that area to the normal.

Evidence of mitosis was observed in six days. Cells that appear degenerated are also present. There is intracellular and intercellular edema. The basal cell layer and the prickle cell layer have regenerated, but the upper strata of epithelium showed a lack of cells resulting in a surface defect in this area (Plate IX, Fig. 13).

By fourteen days the epithelial proliferation has restored the gingival epithelium to the preoperative status (Plate XVI, Fig. 21).

The presence of the keratin layer in the epithelium was evidence of maturation of this tissue.

B. Connective Tissue Production

1. Connective Tissue Fusion

After forty-eight hours an advanced state of fusion has occurred in the wound area. At the gingival zone of this wound, a matrix or stroma is seen that exhibits a pattern of horizontal layers of fibrin coagulum which binded the flap to the fixed, remaining connective tissue (Plate IV, Fig. 7). The spaces between these strands are filled with polymorphonuclear leukocytes. There were also spaces here and there containing a large clot of red blood cells. The entire incision area was occupied by this clot at this time interval.

The bordering areas of the wound showed what appeared to be vascular endothelial cells and young dark staining fibroblasts projecting into this clot or fibrin network.

Budding capillaries were seen in the clot and also projecting from around the severed collagen bundles along the wound edge. New collagen fibers were being laid down as the fibrin threads were resorbed.

While diffusely extravasated blood was quickly and completely absorbed, a large circumscribed collection of blood clot in the tissues cannot be disposed of easily. The space occupied by this clot was a break in the tissues, and it was into this space that young reparative tissues grew from the periphery, penetrating the clot and replacing it. This process was actually the organization of the clot. The organizing tissue was similar to granulation tissue. It consisted of advancing arcades of capillary blood vessels with proliferating fibroblasts and wandering phagocytes to remove debris. This organizing tissue later became less vascular and more fibrous.

2. Connective Tissue Proliferation

In six days the repair phenomenon had advanced considerably. The incision area at the gingival zone of the flap was filled with numerous young fibroblasts (Plate VII, Fig. 11). New bundles of collagen, although disoriented, were found in this area. Some histiocytes also were seen.

In the supra alveolar zone of the incision area the connective tissue has not advanced as much in its repair. One witnesses a gradual rate of repair relative to the region of the wound area (Plate VIII, Fig. 12). Many fibroblasts were seen in the supra alveolar zone, along with some histiocytes. The formation of new collagen bundles lagged behind that of the gingival zone area. The tissue here was more characteristic of granulation tissue.

Much of the same may be said for the stage of repair in the alveolar zone of the incision area.

The orientation of capillaries at this time was similar to the collagen fibers which were parallel with the vestibular surface of bone, rather than perpendicular to the surface of the bone.

The periosteum showed important reactive changes (Plate XII, Fig. 16). Its cells were differentiating, and newly formed blood vessels were developing in it along with many reserve cells. This increased periosteal activity results in the histogenesis of osteoclasts and later osteoblasts (Plate XIII, Fig. 17) (Plate XVIII, Fig. 24).

This periosteal activity was associated with connective tissue proliferation. The endosteal or medullary tissues of the bone showed cellular proliferation.

C. Inflammation

In forty-eight hours the gingival zone of the flap showed capillaries which were not dilated or congested with blood cells. The lamina propria showed many polymorphonuclear leukocytes throughout the time (Plate IV, Fig.7).

The supra alveolar zone of the flap presented capillaries that were normal in size. There was much cellular activity in the periovascular area. These cells were undifferentiated mesenchymal cells, macrophages and reserve cells.

The alveolar zone of the flap showed some congested capillaries and connective tissue which was dense with polymorphonuclear leukocytes and macrophages.

In the gingival zone and the alveolar zone, there was an increase in the number of young fibroblasts.

In the two day specimen there was some congestion and dilation of capillaries in the periosteal connective tissue with a moderate infiltration of polymorphonuclear leukocytes and macrophages in the surrounding tissue.

At no time was an inflammatory response observed in the bone marrow spaces.

III Reconstructive Stage 0-60 days

A. Reconstruction of Connective Tissue

1. Continued Connective Tissue Proliferation 14-21 days

In fourteen day specimens, the connective tissue in the gingival zone of the incision area was young connective tissue (Plate XVI, Fig. 21). Some bundles of collagen fibers were acquiring a wavy pattern of the more mature bundles. They were observed in a pattern continuous with the preexisting bundles on either side of the wound area.

The supra alveolar zone presented this young connective tissue with

mitotic figures and fibers with a disoriented pattern. Here the fibroblasts and the fibers were in a linear direction parallel with the vestibular plate. Many more fibroblasts were seen here than in the gingival zone. This description is similar for the alveolar zone of the incision except it appears behind the supra alveolar zone in organization.

Within twenty-one days, the quality of the connective tissue showed maturity by exhibiting dense bundles of fibers and few cells (Plate XVII, Fig. 23).

2. Connective Tissue Maturation 6-60 days

The connective tissue during this healing period has presented layers at various stages of maturation. In six days the connective tissue was grossly disorganized with young fibroblasts, new collagen fibers, and bundles of fibers placed in all directions with no pattern to their position (Plate VII, Fig. 11).

At fourteen days another stage of maturation displayed itself with the fibroblasts being fewer in number, not as darkly stained nuclei, and bundles aligned to a certain pattern (Plate XVI, Fig. 21). At this time all the connective tissue elements were aligned in a linear direction parallel with the surface epithelium. Of particular importance is also the fact that a gradient of repair existed relative to the connective tissue. More advanced repair had taken place in the subepithelial area of the gingival zone than in the areas parallel with the vestibular bone.

In twenty-one days the quality of the connective tissue matured to the point where there were many collagen bundles, few cells, and dense wavy bundles (Plate XVII, Fig. 23). However, the pattern has remained with the direction of

the bundles being parallel with the surface of the alveolar bone.

In sixty days there was a reorientation of the cellular component and the collagen bundles (Plate XIX, Fig. 26). The pattern shows the bundles being perpendicular to the vestibular plate. Although this pattern was not identical with the control, this connective tissue was compatible with mature connective tissue.

B. Reconstruction of Bone

1. Osteoclastic Resorption 2-21 days

Two days after surgery, an internal resorption took place in the large marrow spaces beneath the vestibular plate in the region of the alveolar zone of the incision. In this alveolar zone some empty lacunae were seen in the bone. A few osteoclasts had differentiated in the connective tissue area adjacent to the acellular necrotic bone tissue found in the outer lamellated portion of alveolar bone (Plate VI, Fig. 9).

However, it was not until six days that a large number of multinucleated giant cells could be seen along the vestibular plate of bone (Plate XII, Fig. 16). Many empty lacunae were found in the vestibular plate (Plate XI, Fig. 15). This was in the period of intense osteoclastic activity. The differentiation of the multinucleated giant cells has taken place in the connective tissue adjacent to the bone (Plate XIII, Fig. 17) (Plate XIV, Fig. 18).

From six to fourteen days osteoclastic activity declined. The marrow space reveals evidence of osteoblastic activity at this time.

The scalloped configuration observed on the surface of the vestibular plate was filled with young connective tissue, capillaries, fibroblasts, fibers, and undifferentiated mesenchymal cells. (Plate XV, Fig. 19).

2. Stage of Osteogenesis 6-60 days

The first sign of osteoblastic activity occurred within six days after surgery. Some of the marrow spaces had osteoid tissue lined with osteoblasts (Plate X, Fig. 14). Other spaces showed endosteal bone formation in a trabecular pattern. Along some of these trabeculae there was osteoid tissue.

However, the first new bone formation along the alveolar crest zone and vestibular plate area was observed at twenty-one days (Plate XVIII, Fig. 24). This area showed coarse fibrillar bone and osteoid tissue.

This new tissue was limited to the alveolar crest area, the vestibular plate side near the crest and the side of the crest facing the periodontal ligament space.

The next time interval of observation was at sixty days and osteogenesis was completed at this time. All other related tissues exhibited complete repair at this time (Plate XIX, Fig. 26) (Plate XXIV, Fig. 36).

CHAPTER V

DISCUSSION

MECHANISM OF REPAIR

I Comparative Study of Two Different Experimental Surgical Procedures Namely, Mucogingival Surgery and Split Thickness Gingival Flap Surgery.

This investigation was conducted to study the specific reaction of bone tissue, connective tissue, and epithelium in the split thickness gingival flap operation. This operation consisted of making a flap with two vestibular incisions, one mesial to the first premolar and the other distal to the fourth premolar. In making the flap, the attempt was made to strip the complete epithelial attachment, split the fibrous connective tissue attachment to the tooth without exposing the root, and continuing the incision to the unattached gingiva without exposing the vestibular plate of bone. Henceforth this operation will be referred to as the split thickness gingival flap surgery.

An investigation to study the histological repair of the oral mucosa and the dento-gingival junction after resection of a muco periosteal flap with exposure of the vestibular bone was completed by Wilderman (1958). His study involved a complete exposure of the bone in the premolar area. This operation will be referred to as mucogingival surgery. The result was traumatic with an extensive loss of alveolar bone occurring until all the exposed vestibular and crestal bone was resorbed. Osteoclastic activity began during the second day and increased to a maximum between four and six days and declined thereafter. During the later stages of osteoclastic resorption, osteoblastic activity had begun and this was during ten days postoperatively. This activity continued

until the greatest bone formation occurred twenty to twenty-eight days. The height of osteoclastic activity occurred during the sixth day postoperatively and the height of osteoblastic activity occurred during the twenty-first day postoperatively.

The height of the alveolar crest after mucogingival surgery was at a lower level than preoperatively. The operation left exposed 4-5 mm. of bone which was completely resorbed by osteoclastic activity from within the marrow spaces and periodontal ligament space. After repair had taken place only 2.5 mm. of the 4-5 mm. alveolar bone previously resorbed had regenerated.

Wilderman reported that the dentogingival junction exhibited a functional repair but with an anatomic deformity. His microscopic findings show changes in the fibrous connective tissue attachment and the epithelial attachment after mucogingival surgery.

The epithelial attachment was attached in a new position which was apical to the position where it previously existed.

The epithelium itself showed changes with respect to the ridge formations. They varied from the normal by exhibiting longer ridge formations.

The fibrous connective tissue attachment of the tooth had doubled its length of attachment to the root.

Wilderman concluded from his findings that there was a functional repair of the dento-gingival junction with an anatomic deformity.

In the study of healing of split thickness gingival flap surgery which is the experimental study reported in this thesis, there was a functional repair with no anatomic deformity.

The bone tissue was not exposed in split thickness gingival surgery.

However, osteoclastic resorption did occur. The resorption took place to a minimum degree in the marrow spaces, but the major portion of resorption took place in the area of the circumferential lamellae of the vestibular plate and alveolar crest area.

Resorption of the alveolar crest and of the vestibular plate was the result of osteoclasts which had differentiated in the periosteum. The height of osteoclastic activity was during the sixth day.

In six days osteoblastic activity was observed in the marrow spaces. This bone formation was considered a response to strengthen the vestibular bone from within the marrow space to counteract the osteoclastic resorption on the outer vestibular plate. In structure it appeared to be an osteophytic bone tissue as described by Weinman and Sicher. It was an immature coarse fibrillar bone tissue.

The major osteoblastic activity occurred during twenty-one days after surgery. Newly formed osteoid tissue was seen at the alveolar crest and along the vestibular plate of bone.

The next specimens at sixty days showed that there was a complete restoration of the alveolar crest to preoperative dimension and relationship to the dento-gingival junction.

An interesting observation at this time is the similarity of peak activity of osteoclastic resorption and osteoblastic formation in both experimental studies with dogs. In the mucogingival surgery experiments with exposed bone, osteoclastic resorption occurred two to ten days and osteoblastic formation resulted in ten to twenty-eight days after surgery. The peak activities were at six and twenty-one days respectively. Correspondingly, in the split thick-

ness gingival flap surgery with the flap repositioned and no exposure of bone, osteoclastic resorption was observed two to fourteen days and osteogenesis six to twenty-one days. The peak activities were six and twenty-one days respectively. In comparing the total formation and resorption activity of both experimental investigations, Wilderman's study showed osteoclastic activity began at the same time but the osteoblastic formation began four days later.

The fibrous connective tissue attachment showed no dimensional change after split thickness gingival surgery compared to the preoperative status.

The epithelial attachment in fourteen days had regenerated in form and position relative to where it was preoperatively.

There was no anatomic deformity after split thickness gingival surgery and the dimensional measurement of the distance of the alveolar crest to epithelial attachment and the length of fibrous connective tissue attachment was the same as in the control specimen.

The question arises as to why the great variation in the amount of bone tissue resorbed in the two studies and the altered repair of one compared to a complete restitution in the other.

It may be concluded at this point that the answer lies in the surgery performed. In the one study, there was a mucogingival flap made and 4-5 mm. of vestibular bone exposed and purposely left exposed for healing to commence from that point. Tissue damage and infection was present. Repair could not occur until there was complete reorganization of the existing tissues. This entailed removal of bone tissue, that had an exposed and infected surface, by an undermining osteoclastic process. The repair was extensive and required

a longer time of action for each phase of repair. By contrast, the split thickness gingival flap results in less damage and repair without deformity.

It is significant to note that the response of the bone, namely osteoclastic resorption and osteoblastic formation, is similar in the two studies despite the variance in the degree of injury. The timing is very similar in both investigations. In the one osteoclasts are active two to ten days, and the other two to fourteen days. The osteoblasts function in one study between ten and twenty-eight days, while the other six to twenty-one days. There is a definite coinciding osseous activity in both experiments.

The degree of activity in the repair response varied in both studies, but the initiation of particular cellular activity occurred at approximately the same time.

The rate of repair in both studies paralleled each other with exception, the epithelium. In Wilderman's work, all epithelium was excised and only after removal and partial restitution of the bone tissue could the epithelium repair. Whereas, the epithelium was the first to respond with the split thickness gingival flap surgery. It is significant to note that this surgery healed by primary intention while the mucogingival flap healed by secondary intention.

The only difference between the two studies in cells is the increased number of cells working in the osteoclastic and osteoblastic phases after the mucogingival surgery. There are many more osteoclasts, and the same with osteoblasts in action during their respective times of activity. This again is related to the degree of damage or trauma by surgery.

From a comparison of these studies one may draw implication that certain

periodontal surgical procedures should be performed in two stages rather than the one procedure as is most often employed at this time.

The primary surgery would be instituted using a split thickness gingival flap procedure. After surgery, a time lapse equivalent to twenty-one days would pass before the next surgery would be performed. The hypothesis that after surgery, there is a mobilization and follow up of cellular activity. Part of this action results in a formation of osteoid tissue over the vestibular and crestal bone. It is at this time that a secondary, more definitive surgery would be performed to benefit from the stage of progressing activity and the protective covering of osteoid tissue over the alveolar crest. It is concluded at this point that bone formation would continue provided that no complete exposure of vestibular bone had occurred during surgery.

It may be possible to compare this to dentin and its behavior after injury. If trauma is introduced to the outer areas of dentin of a tooth (i.e. cavity preparation), it acts as a stimulus to the formation of more dentin. Rather than resorption along the dentinoid or pre-dentin area lining the inner borders of the dentin in the pulp chamber, there is the opposite reaction which is secondary dentin formation.

II Role of the Reserve Cell in Repair.

In this experimental study increased reserve cell activity took place before permanent repair occurred. The reserve cell was a precursor cell for osteoclasts, osteoblasts, fibroblasts and histiocytes. It is an uncommitted, multipotential cell, that is able to differentiate into any specific cell that might be demanded by the tissue environment.

Normally, these cells are always present in connective tissue. However,

when repair of a tissue becomes necessary after damage, these undifferentiated cells tend to increase in numbers. This initial stage of proliferation is soon followed by a stage of specific differentiation into osteoblasts, osteoclasts, and any other type of connective tissue cell.

A) Stage of Proliferation

In this experimental study it was observed that the reserve cells underwent proliferation in the first forty-eight hours after surgery. It was during this time of the initial preparatory stage of healing that these cells multiplied many times. It might be speculated that wound closure mediated by the anchoring clot was a temporary measure. This time period allowed for proliferation of these reserve cells to set the stage for permanent repair. Within the forty-eight hours the dense fibrous connective tissue of the entire surgical area disappeared and it now became highly cellular due to the proliferation of reserve cells. However, this proliferation continued without interruption throughout the entire period of repair.

B) Stage of differentiation

As the reserve cells became available in adequate numbers, they began differentiating into specific cells. Initially, in this experiment, the reserve cells differentiated into osteoblasts. The greatest number were observed in the marrow spaces. In contrast the differentiation of reserve cells along the outer vestibular plate took the form of osteoclasts.

Finally, fibroblasts differentiated from these reserve cells in the connective tissue and complete repair of the hard and soft tissues occurred. There was always a general overlapping of activity of osteoclasts, osteoblasts and fibroblasts during healing. After the wound was repaired, proliferation

of reserve cells ended and the number of these cells in the connective tissue returned to normal.

In conclusion then, this experiment has demonstrated the role of the reserve cell in the healing of a split gingival flap. The reserve cell is a multipotential, uncommitted cell located in the perivascular area. After proliferation it is able to differentiate into osteoclasts, osteoblasts, macrophages, and fibroblasts.

This animal study was conducted on young dogs. It might be of interest to observe reserve cell activity and repair in older animals or animals of different species.

CHAPTER VI

SUMMARY AND CONCLUSION

This investigation was conducted to carefully delineate the processes of healing in the split thickness gingival flap.

Four adult mongrel dogs with fully erupted permanent teeth were used as subjects. Surgery was performed in the region of the maxillary and mandibular premolar teeth. The animals were sacrificed at zero hour, two days, six days, fourteen days, twenty-one days, sixty days.

Surgery of a split thickness gingival flap involved the reflection of the gingiva from the periosteum by delicate dissection allowing no area of bone to be exposed. The flap then was repositioned and healing commenced by primary intention.

The early hours showed a fusion of the flap with the remaining fixed connective tissue by means of a fibrin coagulum. Inflammation took place immediately and continued through six days. Reserve cells had proliferated at this time and continued to proliferate throughout the healing period.

After forty-eight hours the epithelium responded by increased activity to repair the wound defect. In six days the epithelial regeneration was accomplished. At the same time, the connective tissue was highly active and differentiation of many cells occurred. Lymphocytes, phagocytes, fibroblasts, osteoclasts, osteoblasts, and endothelial cells were numerous and mobilized to bring about repair of this split thickness flap.

Ostoclastic resorption was exhibited most during the six days postoperative time. After that their activity diminished until their complete disap-

pearance between fourteen and twenty-one days.

Osteoblastic activity was observed in the marrow spaces two days after surgery. The result was the formation of osteophytic bone to compensate for the loss on the vestibular plate side. However, the intense osteoblastic bone formation was evident at twenty-one days in the vestibular plate and crestal areas.

In sixty days all tissues had repaired themselves and the result showed no anatomic deformity. All healing of the tissues was restored to a preoperative status.

CHAPTER VII

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CHAPTER VIII

APPENDIX

- 1) Clinical photographs
- 2) Photomicrographs
- 3) Diagrammatic illustrations

PLATE I

Figure 1

Clinical photograph of the preoperative premolar area.

Figure 2

Photomicrograph of the control specimen. (X43)

Note:

- 1) Normal continuity of epithelium, lamina propia, and periosteal connective tissue

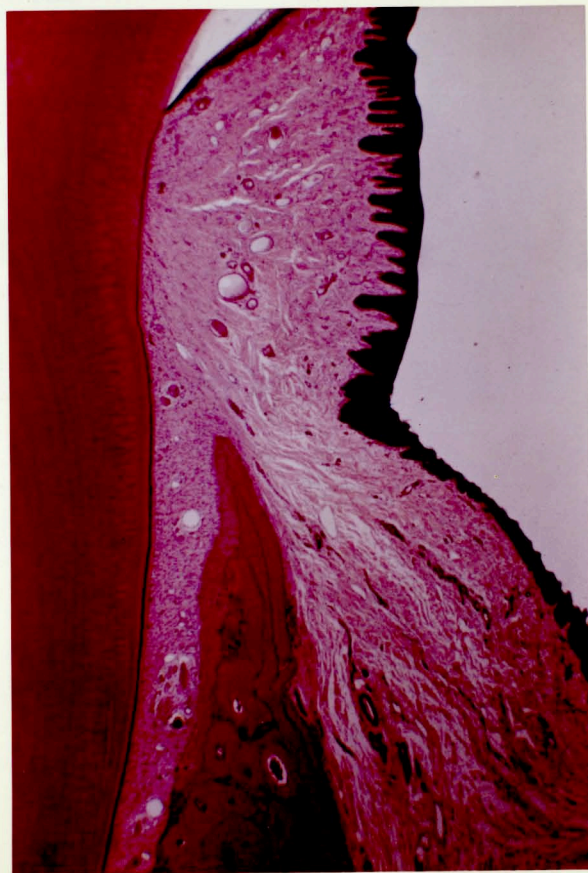


PLATE II

Figure 3

Clinical photograph of the surgical flap in the premolar area demonstrating the periosteal connective tissue remaining on the bone.

Figure 4

Photomicrograph of the 0-Hr. specimen. (X43)

Note:

- 1) Remaining connective tissue still attached to the tooth and periosteum and the remaining tag of epithelial attachment
- 2) Clot between flap and the remaining attached connective tissue
(The space seen is an artifact due to shrinkage)
- 3) Compact vestibular plate of bone

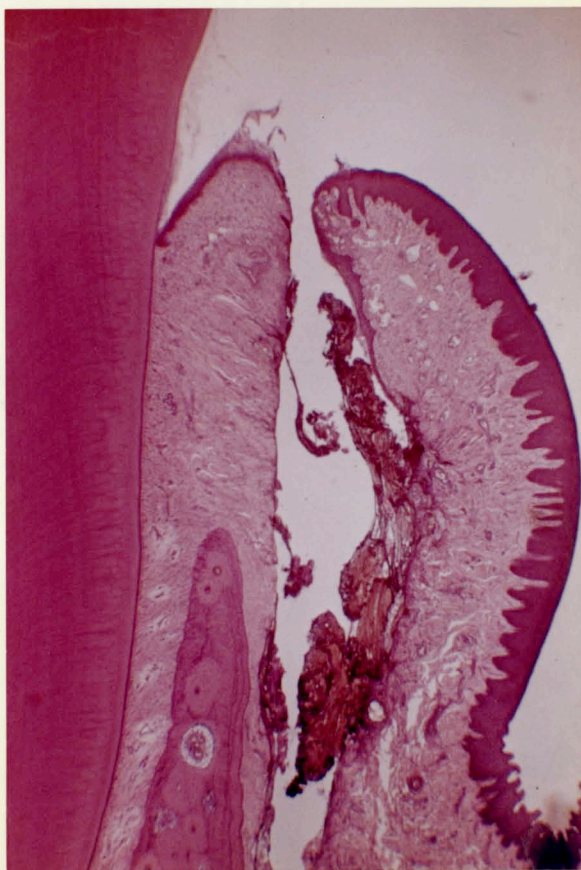


PLATE III

Figure 5

Clinical photograph of the premolar area of operation with split flap sutured in position



PLATE IV

Figure 6

Clinical photograph of operated area two days after surgery.

Figure 7

Photomicrograph of 2 day postoperative specimen. (X43)

Note:

- 1) Fibrin coagulum occupying superficial incision area

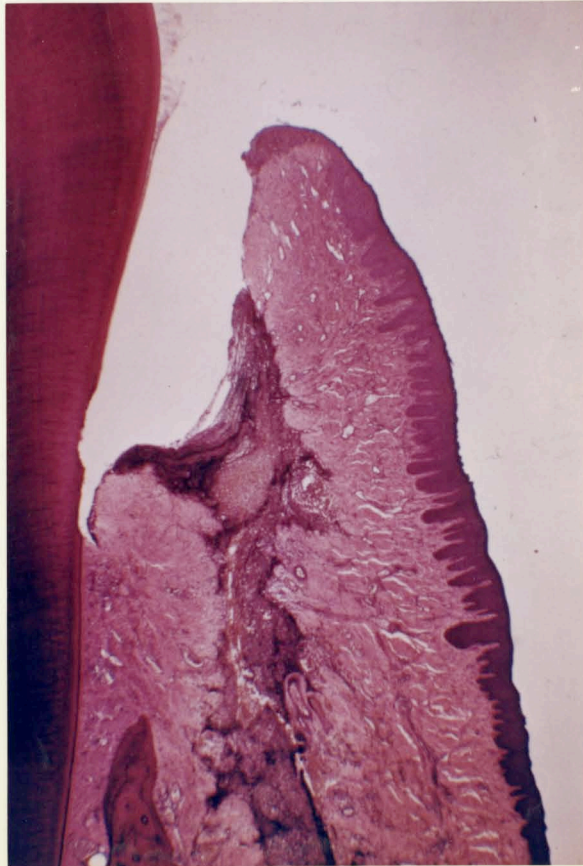


PLATE V

Figure 8

Photomicrograph of superficial incision area 2 days after surgery.

(X43)

Note:

- 1) Fibrin in clot (anchoring clot) (space visible is an artifact)
- 2) Infiltration of polymorphonuclear leukocytes

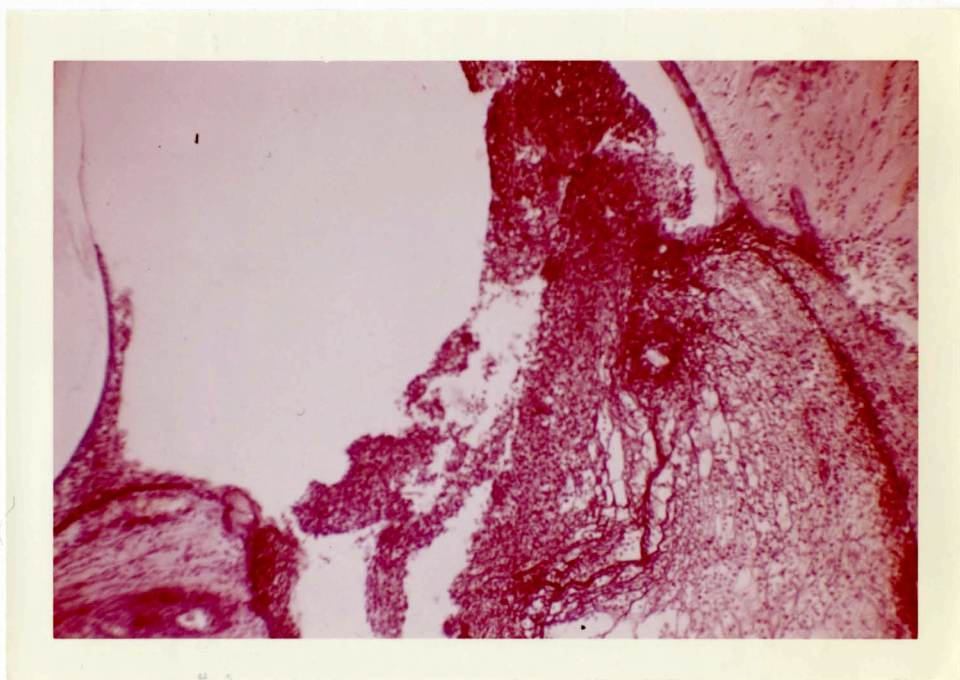


PLATE VI

Figure 9

Photomicrograph of 2 day postoperative specimen. (X100)

Note:

- 1) Periosteal connective tissue covering vestibular plate of bone
- 2) Proliferating reserve cells from marrow space

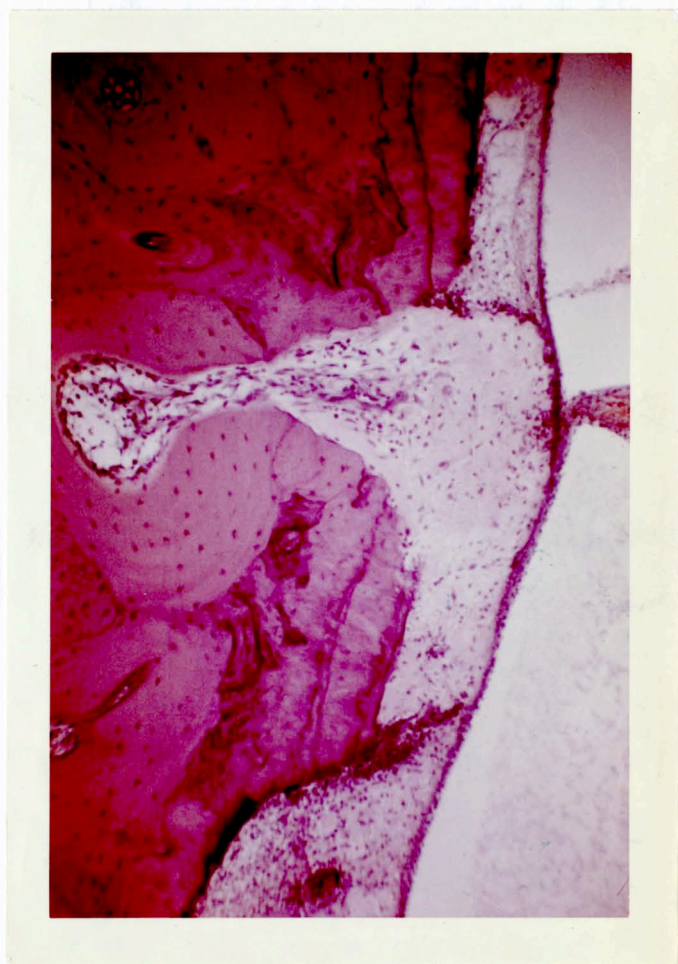


PLATE VII

Figure 10

Clinical photograph of the operative site 6 days after surgery.

Figure 11

Photomicrograph of 6 day postoperative specimen. (X43)

Note:

- 1) Complete fusion of epithelium
- 2) Space at area of incision in alveolar zone is an artifact



PLATE VIII

Figure 12

Photomicrograph of the alveolar zone area 6 days after surgery. (X43)

Note:

- 1) Artifact in incision area
- 2) Increased numbers of reserve cells are noted along line of incision

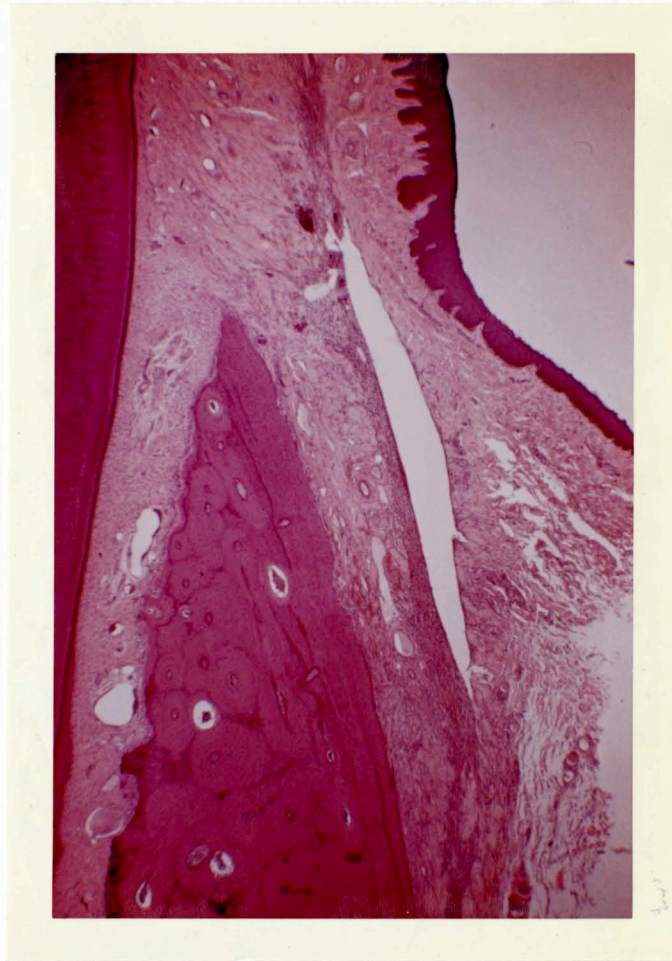


PLATE IX

Figure 13

Photomicrograph of 6 day postoperative area. (X100)

Note:

- 1) Fusion of epithelium
- 2) Basement membrane is continuous
- 3) Lack of cellular continuity in superficial layers of epithelium
- 4) Increase in vascularity and numbers of reserve cells in lamina propia



PLATE X

Figure 14

Photomicrograph of 6 day postoperative specimen. (X100)

Note:

- 1) Resorption on vestibular side
- 2) Endosteal bone formation from marrow side
- 3) Many reserve cells visible in periosteal connective tissue



PLATE XI

Figure 15

Photomicrograph of 6 day postoperative area. (X400)

Note:

- 1) Necrotic bone tissue along vestibular plate area
- 2) Reserve cells in periosteal connective tissue
- 3) Capillaries in periosteal connective tissue

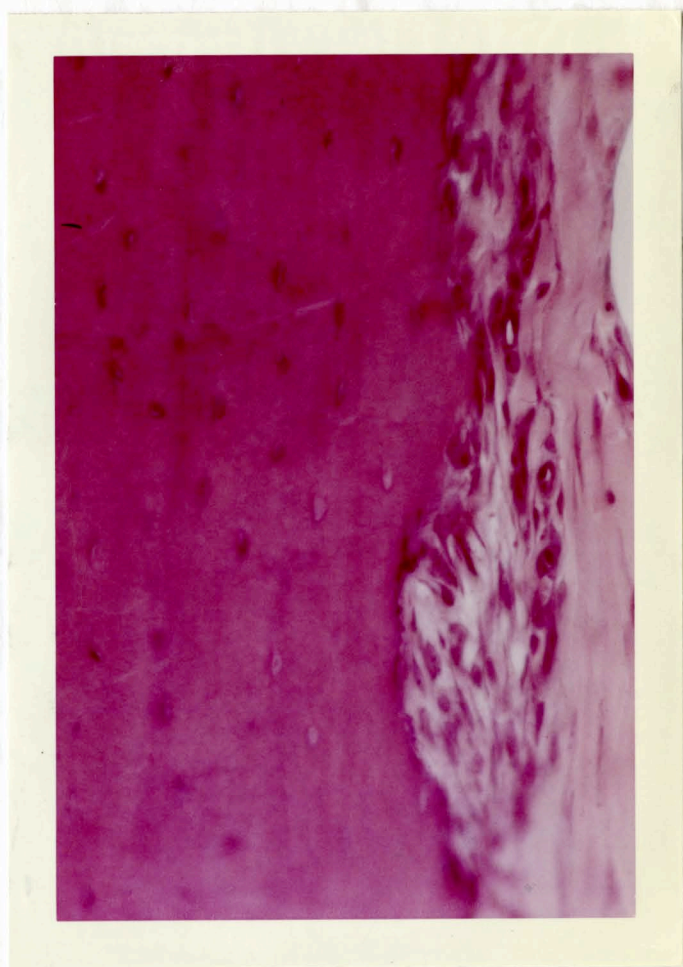


PLATE XII

Figure 16

Photomicrograph of 6 day postoperative specimen. (X400)

Note:

- 1) Empty lacunae of necrotic bone tissue
- 2) Osteoclastic activity
- 3) Note reserve cells
- 4) Vascularity of connective tissue



PLATE XIII

Figure 17

Photomicrograph of 6 day postoperative area. (X400)

Note:

- 1) Necrotic bone tissue
- 2) Osteoclastic activity
- 3) Reserve cells
- 4) Capillaries in connective tissue

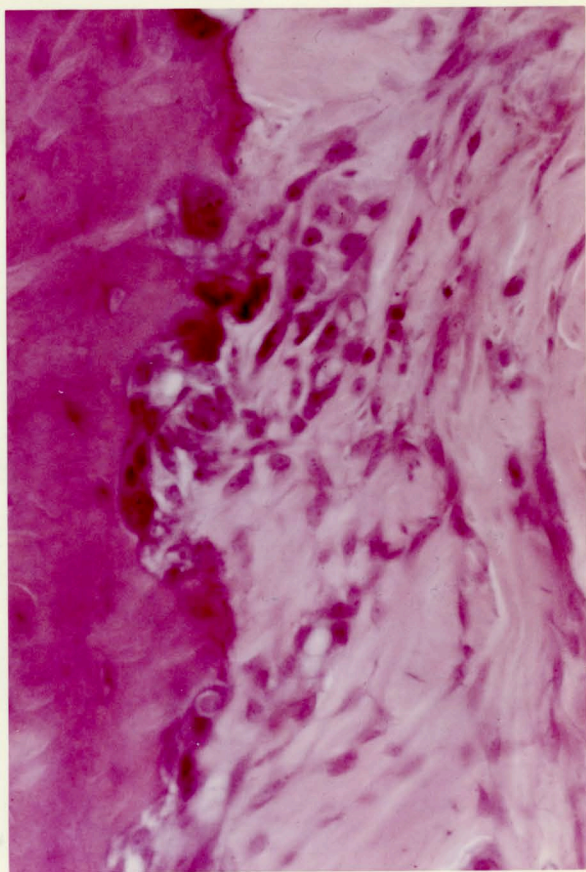


PLATE XIV

Figure 18

Photomicrograph of 6 day postoperative specimen. (X400)

Note:

- 1) Large osteoclasts on vestibular plate side
- 2) Necrotic bone tissue
- 3) Reserve cells in vicinity of capillary

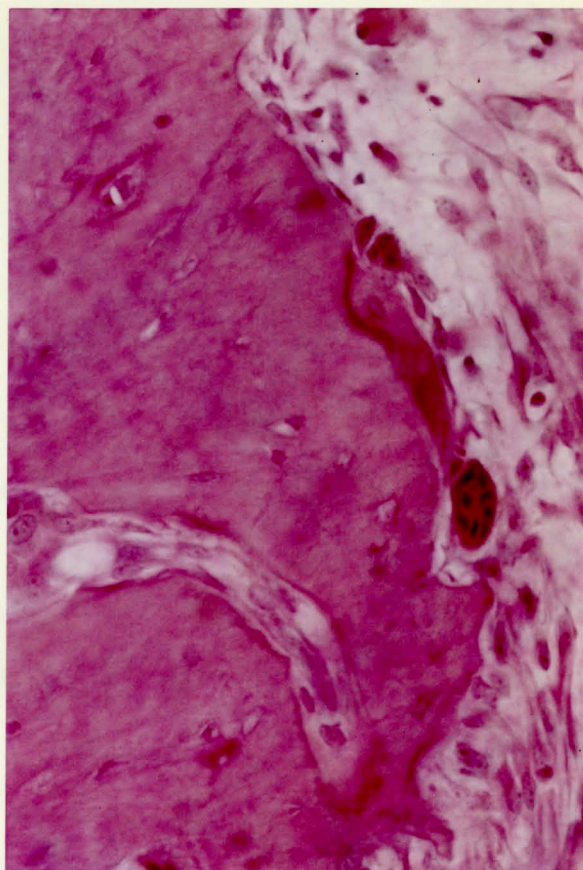


PLATE XV

Figure 19

Photomicrograph of 6 day postoperative specimen. (X400)

Note:

- 1) Necrotic bone in vestibular plate area
- 2) Osteoclasts - (this is period of greatest osteoclastic activity)
- 3) Numerous reserve cells in connective tissue

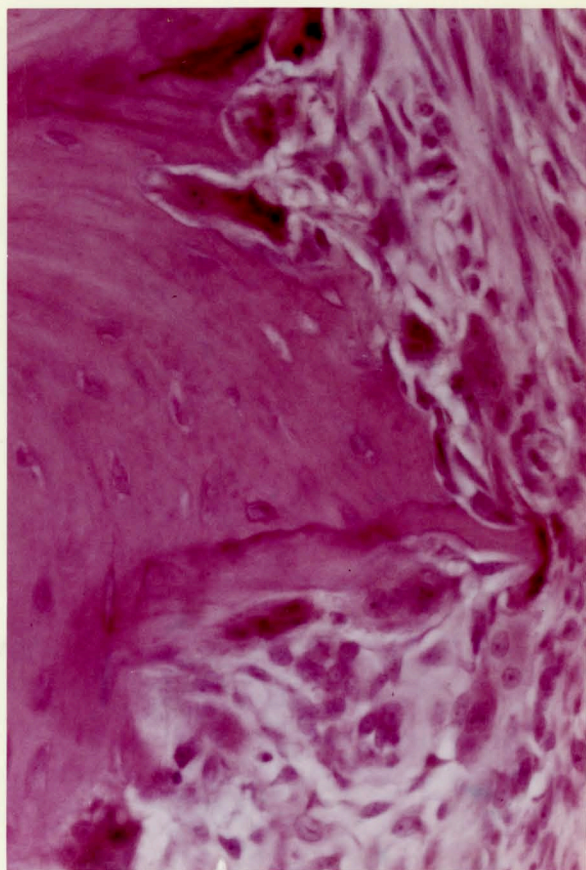


PLATE XVI

Figure 20

Clinical photograph of operated area 14 days after surgery.

Figure 21

Photomicrograph of operated area 14 days after surgery. (X43)

Note:

- 1) Scar tissue visible in previous area of incision
- 2) Decrease in cellularity

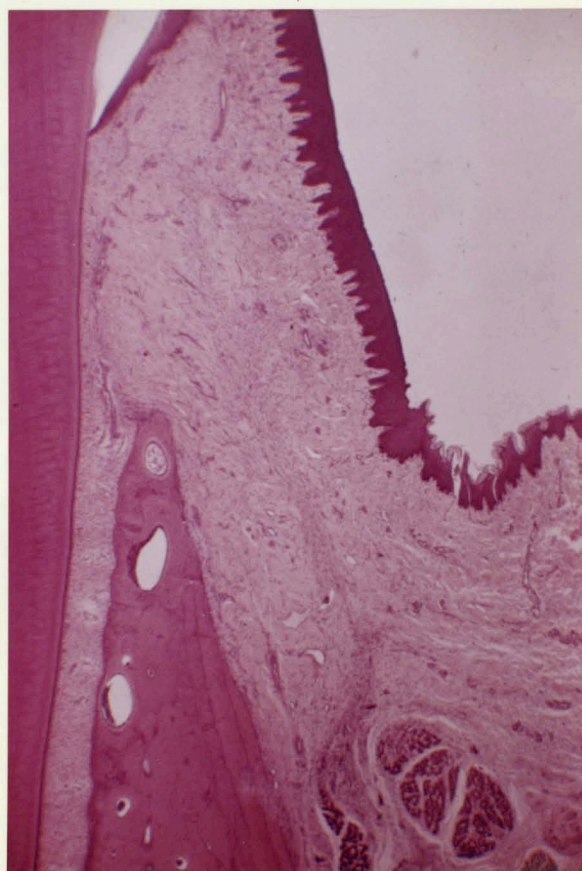


PLATE XVII

Figure 22

Clinical photograph of operated area 21 days after surgery.

Figure 23

Photomicrograph of operated area. (X43)

Note:

- 1) Epithelial attachment
- 2) Connective tissue organization more complete

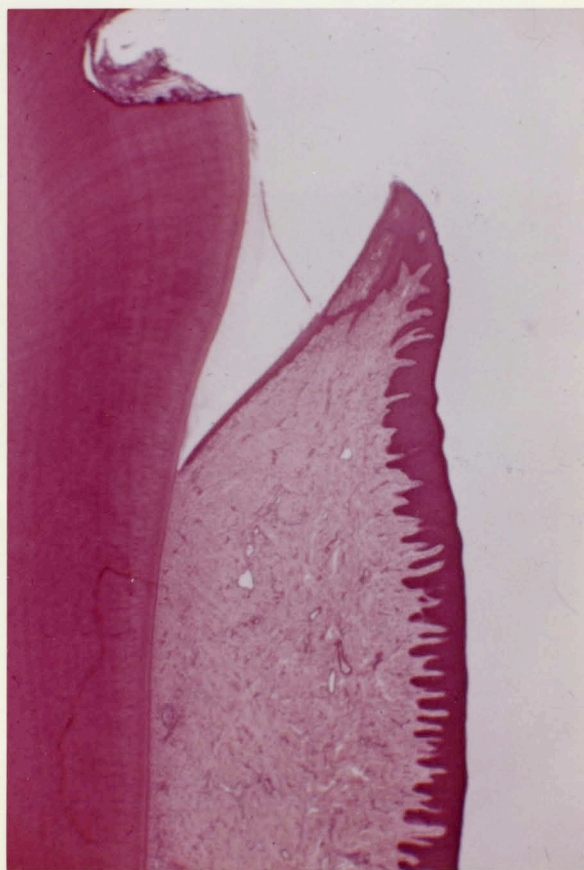


PLATE XVIII

Figure 24

Photomicrograph of the operated area 21 days after surgery. (X100)

Note:

- 1) Osteoblastic activity
- 2) Osteoid tissue along vestibular side and alveolar crest
- 3) Reversal lines
- 4) Cellularity of periosteum along supra alveolar area

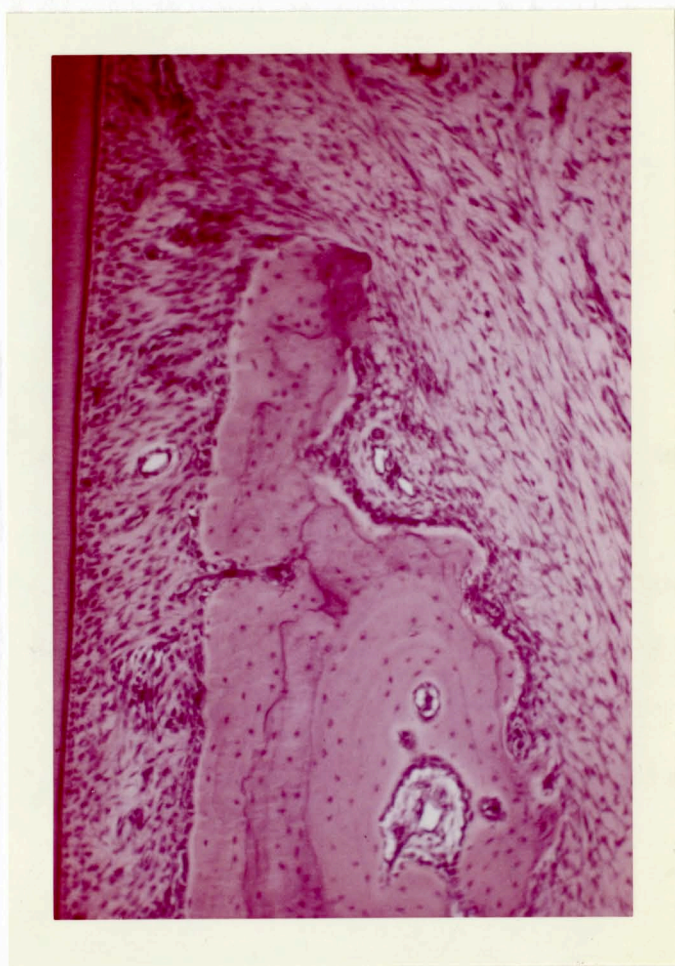


PLATE XIX**Figure 25**

Clinical photograph of operated area 60 days after surgery.

Figure 26

Photomicrograph of operated area 60 days after surgery. (X43)

Note:

- 1) Dento-gingival junction functionally restored without anatomic deformity
- 2) The free and attached gingiva, connective tissue, and alveolar bone are normal

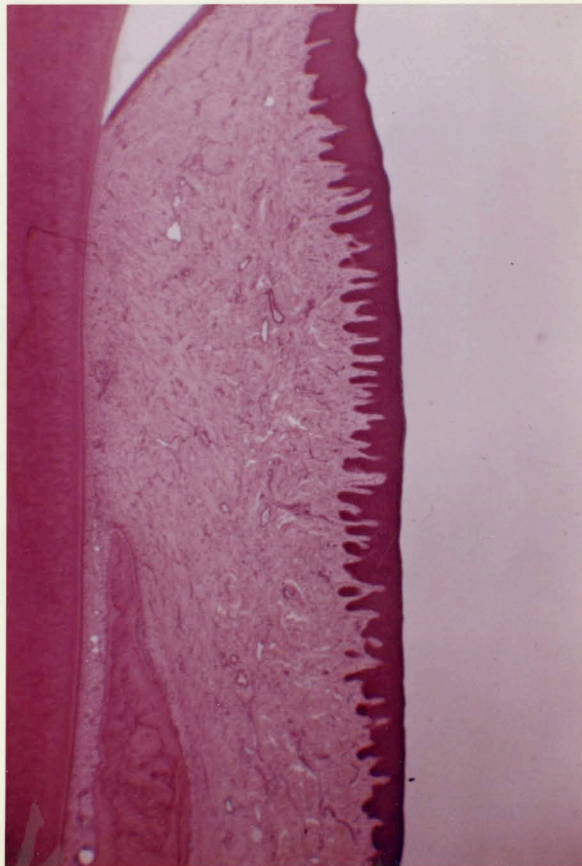


PLATE XX

Diagrammatic illustrations

Figure 27

Diagram of split thickness
flap surgery.

- A - epithelium
- B - remaining stump of
connective tissue
- C - alveolar bone
- D - periosteal connective
tissue covering bone
- E - enamel
- F - clot
- G - remaining tag of
epithelial attachment
- ▨ - tooth

Figure 28

Diagram of three zones
described in the split
thickness flap surgery.

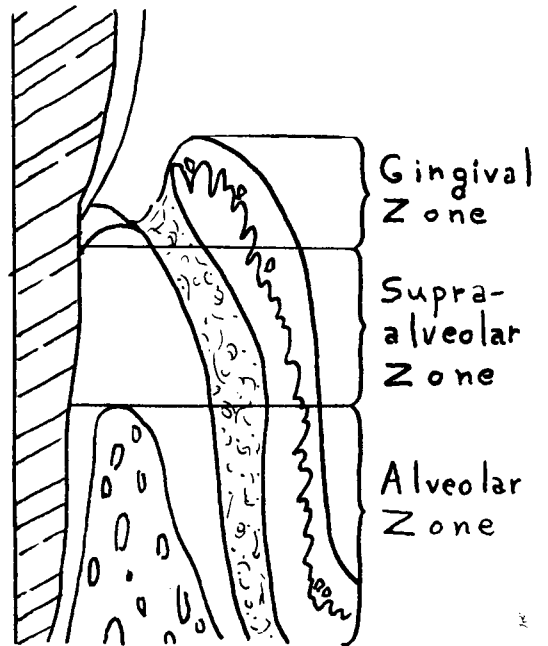
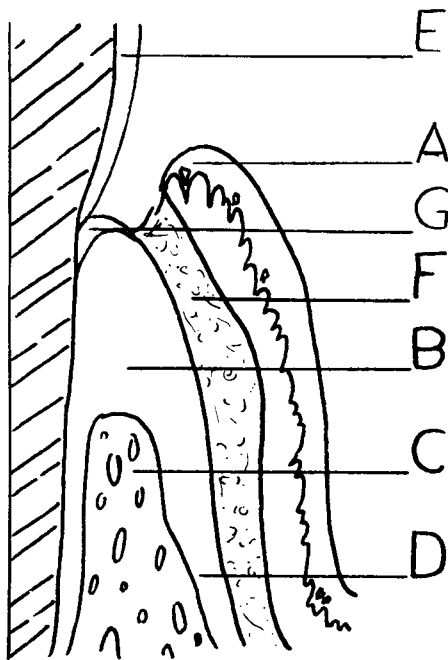


PLATE XXI

Figure 29

Graph comparing osteoclastic activity in two different experiments.

The abscissa is in days while the ordinate is expressed in arbitrary degrees of 1 through 5.

Legend

A - split thickness flap surgery (Staffileno)

B - mucogingival surgery (Wilderman)

Note:

Peak activity coincides in both experiments

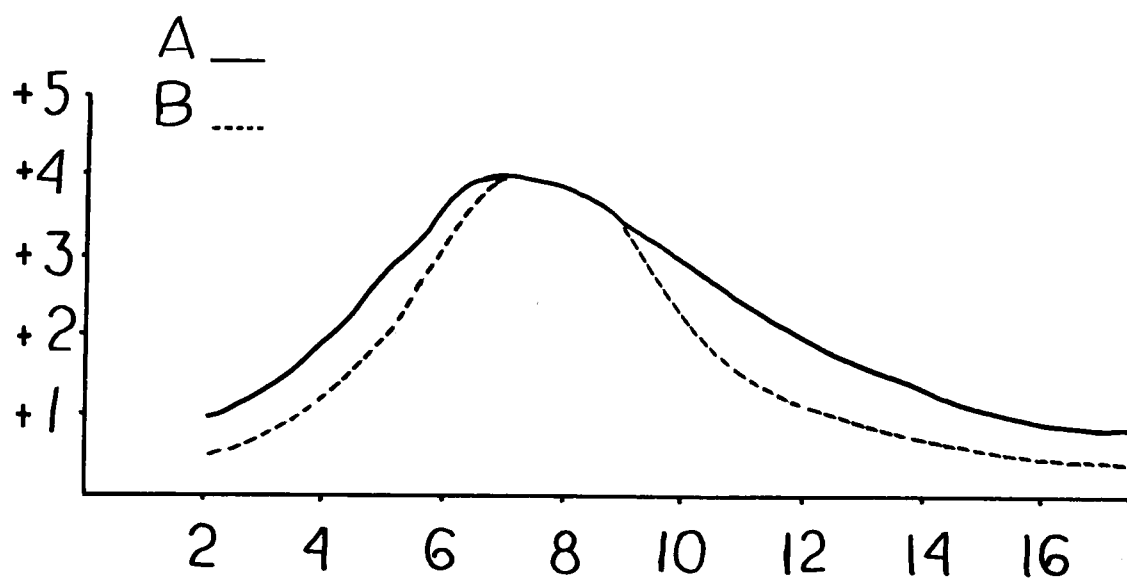


PLATE XXII

Figure 30

Graph comparing osteoblastic activity in two different experiments.

The abscissa is in days while the ordinate is expressed in arbitrary degrees of 1 through 5.

Legend

A - split thickness flap surgery (Staffileno)

B - mucogingival surgery (Wilderman)

Note:

Peak activity coincides in both experiments

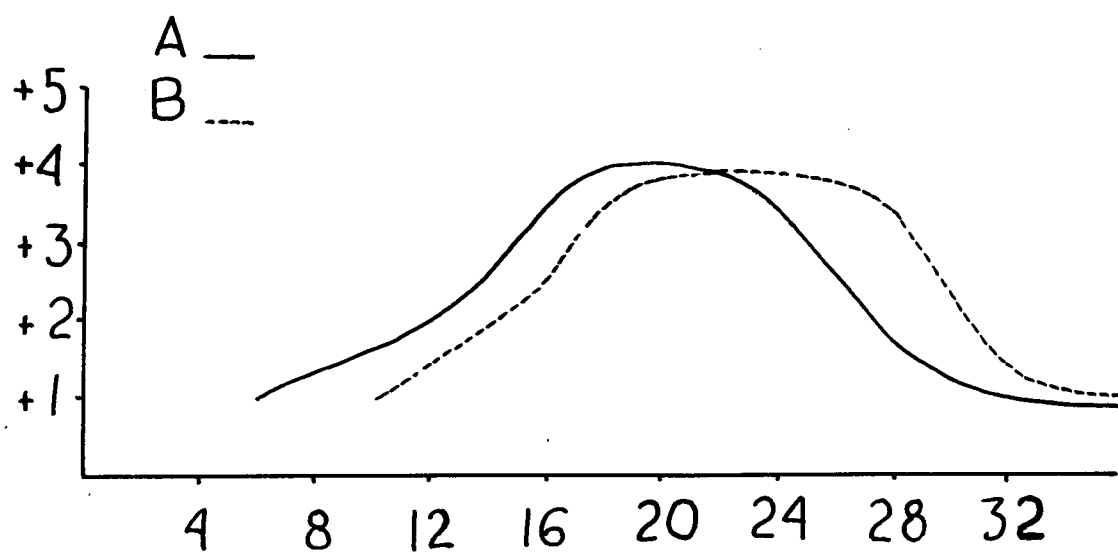


PLATE XXIII


Diagrammatic illustration of mucogingival surgery

Figure 31

Figure 32

Figure 33

Legend

- A - Bone exposure and resorption
- B - New bone formation
- C - Loss of bone
- D₁ - Original connective attachment
- D₂ - New connective attachment
- E₁ - Original epithelial attachment on cemento-enamel
junction
- E₂ - Epithelial attachment apical to cemento-enamel junction
- G - Young connective tissue
- I - Incisions
- X - Epithelium at lower level
-  - tooth

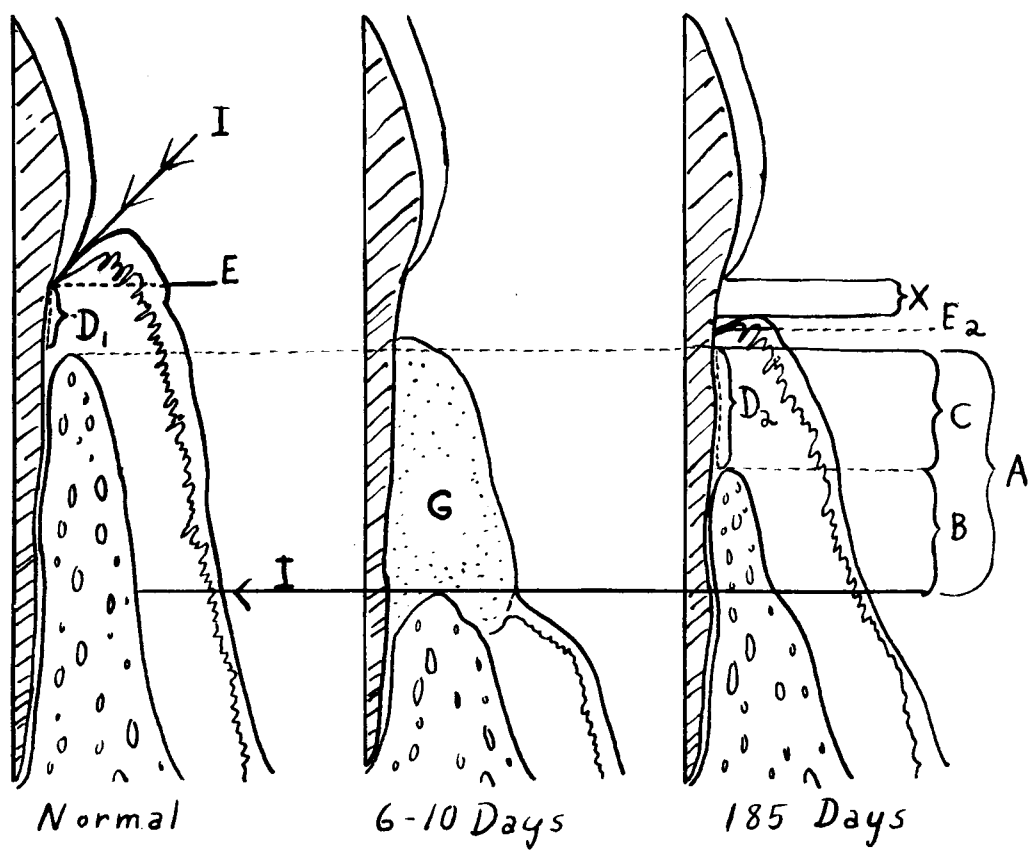


PLATE XXIV

Diagrammatic illustrations of operative area
in split thickness gingival flap surgery

Figure 34

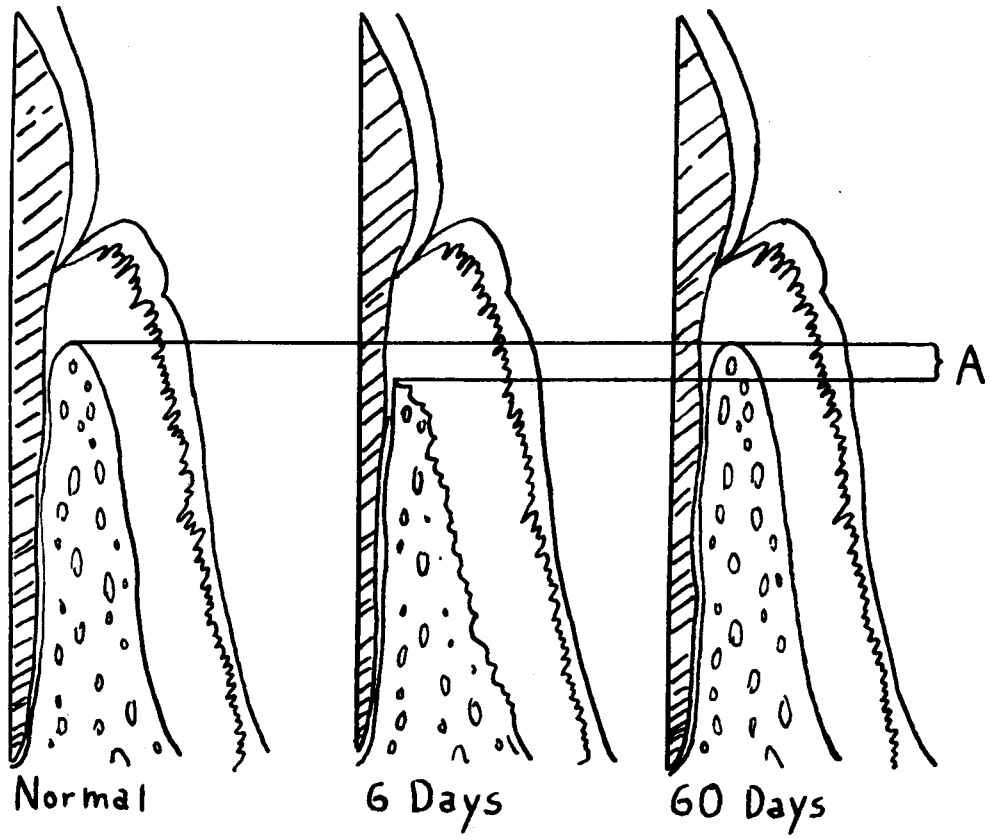
Figure 35

Figure 36

Legend

A - Amount of bone lost 6 days after surgery.

The 60 day diagram shows that after complete healing there was no anatomic deformity.



APPROVAL SHEET

The thesis submitted by Dr. Harry Staffileno, Jr. has been read and approved by three members of the Departments of Anatomy and Oral Anatomy.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

1/13/61
Date

Frank M. Wentz, D.D.S., M.S., Ph.D.
Signature of Adviser